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DOI: https://doi.org/10.1890/15-0599.1

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PREPRINT

The doi for this manuscript and the correct format for citing the paper are given at the top of the online (html) abstract.

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Plant selection and soil legacy enhance long-term biodiversity effects

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Abstract

Plant–plant and plant–soil interactions can help maintain plant diversity and ecosystem functions. Changes in these interactions may underlie experimentally-observed increases in biodiversity effects over time via the selection of genotypes adapted to low or high plant diversity. However, little is known about such community-history effects and particularly the role of plant–soil interactions in this process. Soil-legacy effects may occur if co-evolved interactions with soil communities either positively or negatively modify plant biodiversity effects. We tested how plant selection and soil legacy influence biodiversity effects on productivity, and whether such effects increase the resistance of the communities to invasion by weeds. We used two plant selection treatments: parental plants growing in monoculture or in mixture over 8 years in a grassland biodiversity experiment in the field, which we term monoculture types and mixture types. The two soil-legacy treatments used in this study were neutral soil inoculated with live or sterilized soil inocula collected from the same plots in the biodiversity experiment. For each of the four factorial combinations, seedlings of eight species were grown in monocultures or 4-species mixtures in pots in an experimental garden over fifteen weeks. Soil legacy (live inoculum) strongly increased biodiversity complementarity effects for communities of mixture types, and to a significantly weaker extent for communities of monoculture types. This may be attributed to negative plant–soil feedbacks suffered by mixture types in monocultures, whereas monoculture types had positive plant–soil feedbacks, in both monocultures and mixtures. Monocultures of mixture types were most strongly invaded by weeds, presumably due to increased pathogen susceptibility, reduced biomass and altered plant–soil interactions of mixture types. These results show that biodiversity effects in experimental grassland communities can be modified by the evolution of positive vs. negative plant–soil feedbacks of plant monoculture vs. mixture types.

Key words: biodiversity effects; plant–soil feedbacks; plant productivity; selection; weed suppression.

Introduction

Diminishing species richness reduces ecosystem functioning (Balvanera et al. 2006, Isbell et al. 2011, Cardinale et al. 2012), including most prominently primary productivity. Long-term experimental manipulations of plant biodiversity have shown that higher plant species diversity increases stability and productivity within communities, with an increasing effect size over time (Tilman et al. 2006, Reich et al. 2012). In a previous study we showed that this strengthening of biodiversity effects in experimental communities can occur through the selection for individuals with greater niche differentiation among species in plant mixtures (Zuppinger-Dingley et al. 2014). This adaptation to the local biotic environment may be due to the sorting out of suitable genotypes within species (Stebbins 1969, Bossdorf et al. 2008) by differential survival or, less likely, to the appearance of new genotypes by recombination or mutation. The observed selection took place over 8 years in a long-term biodiversity experiment in Jena, Germany, and led to greater character displacement of functional traits between species, thereby enhancing species complementarity and biodiversity effects (Zuppinger-Dingley et al. 2014). We refer to these plants selected in mixtures as mixture types.

In contrast, we refer to plants selected in monocultures as monoculture types and here hypothesize that they may have increased defense against species-specific pathogens, known to accumulate in monocultures (van der Putten et al. 2013), or increased beneficial interactions with soil mutualists like mycorrhizal fungi or growth-promoting bacteria, which can also accumulate near host plants (Pregitzer et al. 2010). In the longer-term, this may lead to the evolution of positive plant–soil feedbacks (Sanders and Fitter 1992, Pregitzer et al. 2013, Schweitzer et al. 2013, c.f. also Corradi and Bonfante 2012), which can be tested when

monoculture types are grown on soils taken from monocultures of their own species. If mixture types are less well adapted than monoculture types to deal with pathogens, negative plant–soil feedbacks might modify the positive effect of character displacement among mixture types. We could not test this in the study of Zuppinger-Dingley et al. (2014) because plants were grown in soil without a legacy of the studied plant species. Such soil legacy has increasingly been recognized to play a role in species coexistence in mixed plant communities (Bartelt-Ryser et al. 2005), with plant–soil feedbacks recently suggested as drivers of complementarity between plant species (Schnitzer et al. 2011, Eisenhauer 2012, Kulmatiski et al. 2012). The mechanisms driving these feedbacks can be considered analogous to Janzen-Connell effects of aboveground herbivores (Janzen 1970, Connell 1971), whereby diversity of plants is promoted by herbivore or pathogen damage done to concentrated stands of closely related plants, leading to enhanced survival of juveniles at greater distances from their mature parent plants. Such belowground Janzen-Connell effects have been shown to strongly influence grassland plant communities in terms of their productivity and composition (Petermann et al. 2008, van der Heijden et al. 2008). In the broad sense we here refer to plant–soil feedbacks (Bever 1994, Mills and Bever 1998, Zuppinger-Dingley et al. 2011) as soil-legacy effects. Combining these effects with those of plant selection in monocultures vs. mixtures, we hypothesize that plant–soil feedbacks should modify biodiversity effects in the following way (hypothesis 1): mixture types (low pathogen defense) in contrast to monoculture types (increased pathogen defense/beneficial interactions) should suffer in monoculture on soils with live inoculum but not on soils with sterile inoculum. Therefore, mixture types, due to the low performance in monoculture, should show stronger biodiversity effects on soils with live inoculum than on soils with sterile inoculum. This effect should be weaker or not exist for monoculture types.

In view of longer-term plant-selection and soil-legacy effects, it is warranted to investigate how susceptible the plant communities in their respective soils are to invasion by other plant species. Communities with higher species diversity are often considered to be more resistant to invasion by weeds because species-rich communities have less available niche space for invaders to establish (Levine 2000, MacDougall et al. 2009). Biodiversity may therefore play a key role in the resistance of a community to weed invasion (Kennedy et al. 2002, Turnbull et al. 2010). In addition, weeds may benefit from either pathogen release or from symbiotic mutualists (Reinhart and Callaway 2006), contributing to the susceptibility of plant communities to invasion (Klironomos 2002), depending on the relatedness of resident and invasive plant species (van Ruijven et al. 2003). Combined with plant selection history, there are contrasting possibilities how soil legacy may modify plant biodiversity effects on resistance to weed invasion (hypothesis 2): in the absence of soil legacy, that is when aboveground effects dominate, greater niche complementarity among mixture types should leave less available niche space for invaders, thus increasing invasion resistance. However, with soil legacy, susceptibility to weeds is expected to be higher and may be largest for monocultures of mixture types. This would be expected for two reasons: first, the increased niche complementarity may result in reduced niche width of mixture compared to monoculture types (Zuppinger-Dingley et al. 2014), and second, lowered pathogen defense may further weaken monocultures of mixture types on soils inoculated with monoculture soil.

In the present study, we investigated the effects of plant selection and soil legacy, as well as their interaction, on biodiversity effects and on the susceptibility to species invasion. Net biodiversity effects were measured by comparing 4-species plant mixtures with the average monoculture of the four species. These net effects were partitioned according to Loreau and Hector (2001) into complementary and sampling effects (we use this term instead of the term ‘selection effects’ to avoid confusion with the term ‘plant selection’ in this paper).

Plant-selection effects were tested by comparing monocultures and mixtures planted with mixture types or with monoculture types, derived from monoculture and mixture plots after 8 years of establishment in a biodiversity experiment in Jena, Germany. Soil-legacy effects were tested by inoculating a neutral background soil with live or sterilized soil conditioned for 8 years in monocultures or mixtures in the same biodiversity experiment. The level of invasion by new species was evaluated by collecting the weeds that appeared in our outdoor experiment at regular intervals and quantified their biomass.

Methods

We used the Jena Experiment, a large grassland biodiversity experiment in Germany (50°55'N, 11°35'E, 130 m a.s.l.) as our source of plant and soil material reflecting 8 years of plant selection and soil legacy. In April 2010 we collected 4900 plant cuttings from monocultures and mixtures in Jena. This plant and soil material was transferred to a new experiment with monocultures and 4-species mixtures in pots in an experimental garden at the University of Zurich, Switzerland (47°33′N, 8°37′E, 534 m a.s.l.). We chose eight out of the 60 grassland plant species occurring in the Jena Experiment. Two species were chosen from each of four plant functional groups: grasses (*Festuca pratensis*, *Poa pratensis*), legumes (*Onobrychis viciifolia*, *Trifolium repens*), tall herbs (*Crepis biennis*, *Galium mollugo*,) and small herbs (*Plantago lanceolata*, *Prunella vulgaris*) (see Roscher et al. 2004 for species nomenclature).

These plant cuttings were used to establish plots in slug-exclosure fences in the experimental garden in Zurich in an identical plant composition to the 48 plots in Jena from which the cuttings had been collected. We added a layer of homogenized potting soil (BF 4, De Baat; Holland) to each plot, which had been filled with field soil without plant growth for 10 years, to ensure that each cutting established well in the new plot. The plots were caged individually with white mesh fabric to minimize potential cross-pollination between plots.

During the summer of 2010 we collected fruits from the cutting-derived plants. We removed the seeds from the fruits and stored them at 10–15 ºC and 50 % humidity in a climate chamber. The seeds were cold-stratified by storing them in a cold room (5 ºC) for four months to simulate a winter period before germinating. Seeds were germinated in a 10.5-h light per day regime with 14–19 °C during the light and 10–16 °C during the dark period.

The seedlings were used to assemble test communities in pots of 16 individuals per pot of 25 × 25 cm area and 25.5 cm depth. Monoculture test communities contained individuals of a single species, whereas mixture test communities contained four individuals each of the four functional groups; that is, all mixtures were 4-species, 4-functional group mixtures. There were eight monoculture species and 16 unique mixture species compositions (Appendix A, Table A1). Each species occurred in eight mixture compositions. Monoculture and mixture test communities were derived from monoculture and mixture selection communities, yielding four combinations of planted-diversity × plant-selection treatments. Plant selection corresponds to monoculture vs. mixture growth history in the Jena Experiment. For each of the eight monocultures and 16 mixture combinations assembled from each of the two monoculture and mixture plant-selection treatments, we planted six replicates into pots in early May 2011; three replicates contained soil with live and three contained soil with sterilized inoculum from the Jena Experiment (the two soil-legacy treatments). *Poa pratensis* selected in monoculture did not germinate well, so monocultures of this species and plant-selection treatment were reduced to four individuals in pots of 11 × 11 cm and 12 cm depth) and two of the planned mixture combinations (*Poa pratensis, Plantago lanceolata*, *Trifolium repens*, *Crepis biennis* and *Poa pratensis*, *Prunella vulgaris*, *Trifolium repens*, *Crepis biennis*) with this species were excluded. One combination (*Poa pratensis*, *Prunella vulgaris*, *Galium mollugo*, *Onobrychis viciifolia*) was replicated in two instead of the planned three replicates.

171 The neutral soil in the pots consisted of 50% agricultural soil (without legacy of the
172 plant species used in our experiment), 25% sand and 25% Perlite (Ricoter AG, Aarberg, Table
173 A2). For the soil-legacy treatments the neutral soil was inoculated with soil inoculum at 4% of
174 the total mass using soil from either monocultures or mixtures in the biodiversity experiment
175 in Jena. To create the Jena soil inoculum, bulked soil samples were taken from the respective
176 plots (eight cores of 0–5 cm depth per plot) from which the plant material had been collected.
177 The soil inocula for the pots containing monocultures were collected from each corresponding
178 original monoculture plot in Jena such that each species was inoculated with soil from that
179 specific species' monoculture soil. Soil inocula for the mixture pots were pooled samples
180 collected from all four-functional group plots in Jena from which plant material was collected.
181 The neutral soil received sterilized inoculum from the respective Jena plots at 4% of the total
182 mass. Inocula were sterilized by autoclaving for 20 minutes at 121°C. To prepare the soil
183 inoculum, the soil samples collected from each plot were homogenized using a 5 mm sieve.

184 Each of the replicate pots was placed in one of the three blocks in the experimental
185 garden in Zurich. Each block contained one pot per treatment combination (planted species
186 identity/composition × plant selection × soil legacy) and was surrounded by a slug-exclosure
187 fence. The treatment combinations were completely randomized within blocks. Seedlings of
188 planted species that did not survive the first two weeks were replaced with spare seedlings of
189 the same species, plant-selection treatment and age. Weeding was carried out throughout the
190 growing season at fortnightly intervals. The weeds were kept, dried and their combined
191 aboveground biomass per pot was determined. Weeds were defined as those species that had
192 not been included among the planted species in the corresponding pot. Weed species included
193 species used in the experiment as well as others.

194 Three weeks after planting, all plants were cut back to 5 cm to standardize their sizes.
195 Fifteen weeks after transplanting, all plants were harvested at 3 cm above ground to determine

196 the aboveground biomass production within the 12-week period late May–mid August 2011.
197 The survival of plants within each community was noted after three and 15 weeks.

198 *Data analyses*

199 We analyzed the proportion of weeds in the total dry aboveground biomass (planted
200 species + weeds) and the log-transformed dry aboveground biomass of planted species as a
201 function of the above-mentioned treatments using mixed-model analysis of variance. The
202 fixed terms in the analysis were planted diversity (monoculture vs. mixture test communities),
203 plant-selection treatment (monoculture vs. mixture selection communities, i.e. monoculture
204 vs. mixture types), soil-legacy treatment (live vs. sterilized inoculum) and interactions among
205 these. Block and species identity/composition were used as random terms. The monocultures
206 of *Poa pratensis* selected in monocultures, which had lower numbers of individuals were
207 excluded from the above analysis.

208 To calculate biodiversity effects for mixture test communities we used the additive
209 partitioning method of Loreau and Hector (2001), which partitions net biodiversity effects
210 into complementarity and selection effects. To avoid confusion with the term selection in the
211 plant-selection treatment, we here use the term "sampling effect" for the second of the
212 partitioned biodiversity effects. The net biodiversity effect is the difference between the
213 mixture and the average of the monocultures of the species making up the mixture. If the
214 different species contribute similarly to mixture biomass, this is reflected in a large
215 complementarity effect. If a few or a single species dominate the mixture biomass, the net
216 effect is reflected in a large sampling effect. The model-predicted mean value was used for
217 *Poa pratensis* monocultures with plants selected in monoculture. Values for net effects,
218 complementarity effects and sampling effects were also analyzed using mixed-model analysis
219 of variance. Here, the fixed terms were plant-selection treatment, soil-legacy treatment and
220 their interaction. Species composition and interactions were used as random terms.

Finally, we calculated soil-feedback effects as the log-ratio of plant aboveground biomass per pot inoculated with live (i.e., "soil legacy") vs. sterilized soil from Jena. Fixed terms were planted diversity (monoculture vs. mixture test communities), plant-selection treatment (monoculture vs. mixture types) and their interaction. Species composition and interactions were used as random terms. Statistical analyses were conducted using the software products R, version 2.15.3 (R Development Core Team, 2013), and GenStat, version 16 (VSN International Ldt. 2013).

To test whether the aboveground biomass of planted species affected the invasion by weeds, we also added it as a covariate to the analysis of the proportion of weeds in the total biomass.

Results

Biodiversity effects (hypothesis 1)

As expected, mixture test communities were more productive than monoculture test communities (Fig. 1a, Table 1, $P = 0.019$). Test communities with live soil inoculum tended to have higher biodiversity net effects than communities with sterilized inoculum (Fig. 2a; Table 2, $P = 0.076$) indicating that soil legacy may strengthen positive plant diversity effects. Indeed, live soil inoculum significantly increased the complementarity effect (Table 2, $P = 0.005$). However, this main effect of soil legacy was driven by an underlying significant interaction between plant-selection and soil-legacy effects, confirming our hypothesis 1 (Fig. 2b; Table 2, $P = 0.048$): the complementarity effect was particularly large in pots containing progeny from plants selected in mixtures (mixture types) and containing live soil inoculum from Jena. In contrast, sampling effects were reduced by soil legacy (Table 2, $P = 0.021$), driven by a similar underlying plant-selection by soil-legacy interaction: the sampling effect was particularly low (even negative) in pots containing mixture types and live soil inoculum (Fig. 2c; Table 2, $P = 0.006$). The number of plants surviving in our experimental

communities did not differ between plant-selection treatments over the 15 weeks of our study (Table B1).

Soil-legacy effects (mechanism underlying hypothesis 1)

In pots inoculated with live soil, but not in pots inoculated with sterilized soil, plant biomass was lower for mixture than for monoculture types (Fig. 1; Table 1, $P = 0.020$). Mixture types experienced overall negative plant–soil feedbacks, while monoculture types experienced overall positive plant–soil feedbacks (Fig. 3a; Table 3, $P = 0.002$). The negative effect of soil legacy for mixture types was stronger in monocultures, where the soil inoculum came from monoculture plots in Jena, than in mixtures, where the soil inoculum came from mixture plots (see in particular the two species *Crepis biennis* and *Onobrychis viciifolia* in Fig. 3b), leading to the significant interaction of planted diversity x plant-selection treatment in Table 3 ($P = 0.046$). The positive effect of soil legacy for monoculture types was equally strong in monocultures, where the soil inoculum came from monoculture plots, and in mixtures, where the soil inoculum came from mixture plots (Fig. 3a). Thus, monoculture types seemed to similarly benefit from feedbacks of live soil inoculum in monocultures and mixtures. Only for two of the eight tested species did soil legacy have a negative feedback on progeny of plants selected in monocultures (*Crepis biennis*, *Trifolium repens*, Fig. 3b). Overall, these results are consistent with the proposed mechanism underlying hypothesis 1.

Weed Biomass in Monocultures vs. Mixtures (hypothesis 2)

Pots containing plant monocultures had a higher proportional weed biomass than pots containing plant mixtures (Fig. 4; Table 4, $P = 0.012$). This effect was particularly strong for monocultures of mixture types inoculated with live soil from Jena (significant 3-way interaction in Table 4, $P = 0.018$); which had a large proportion of weed biomass, as predicted by hypothesis 2.

When we used the aboveground biomass of planted species as a covariate (log-transformed) in the first place of the sequence of fixed effects in the mixed model (table not shown), it was highly significant ($P < 0.001$) and removed the significance of the planted diversity term but not of the three-way interaction. That is the negative effect of the biomass of planted species on the proportion of weeds of the total biomass in a pot was responsible for the observed treatment main effect but could not explain subtle differences due to plant selection and soil legacy.

Discussion

In a previous study we observed that grassland primary productivity can be driven by plant selection in field biodiversity experiments, specifically by the selection for increased niche complementarity between species in mixtures (Zuppinger-Dingley et al. 2014). In particular, this earlier study showed that communities of plants derived from mixture field plots (mixture types) had stronger biodiversity effects than plants derived from monoculture field plots (monoculture types) in neutral soil in the glasshouse. One puzzling aspect of these previous results was that the evolutionarily-increased character displacement of mixture types grown in mixtures was independent of the specific type of mixture in which the plants were selected over 8 years in the Jena Experiment. The evolution to mixture types could have happened in a non-directional way if within-species trait variances decreased without shifts of trait means. Another possibility would be that selection in mixtures was for generally increased plasticity, allowing these plants to achieve niche differentiation plastically in response to the specific mixture in which they were grown.

In the present study, net biodiversity effects on neutral soil in the experimental garden were slightly stronger for mixture than for monoculture types, but complementarity effects increased more for mixture than for monoculture types on soils with legacy (see Fig. 2a, b). The soil legacy treatment was obtained by inoculating neutral soil with soil from monoculture

or mixture plots, thus allowing soil organisms which had accumulated under these plant diversity treatments in the field to associate with these same plant communities in our garden experiment. This legacy treatment led to negative plant–soil feedbacks for mixture types planted in monoculture pots.

Plant selection and soil legacy increase biodiversity effects (hypothesis 1)

Thus, in the present study, mixture compared to monoculture types showed increased biodiversity effects with soil legacy due to reduced monoculture performance, whereas in the previous study (Zuppinger-Dingley et al. 2014) mixture types showed increased biodiversity effects on neutral soil due to increased mixture performance. We currently have no explanation why this latter effect was not observed in the present study. However, combining the results of the two studies suggests that mixture types may increase biodiversity effects over time for two reasons: first, increased niche complementarity and performance in mixtures and, second, reduced pathogen defense and performance in monocultures. In contrast, monoculture types in this study seem to have increased pathogen defense or may have developed a better capability to benefit from mutualists because they performed better in soils with legacy than in neutral soil, reflected in positive plant–soil feedbacks of monoculture types, both when grown in monoculture and mixture (see Fig. 3a). These results support our hypothesis 1: mixture types performed worst in monoculture on soils with live inoculum and thus have increased biodiversity effects, in particular complementarity effects, on soils with legacy compared to neutral soil. However, not all species of mixture types had lower performance in monocultures on soil with legacy than on neutral soil. Since the two exceptions were rather low-yielding species (*Galium mollugo* and *Prunella vulgaris* in Fig. 1b), they may have caused the negative sampling effect for mixture types on soil with legacy (see Fig. 2c). In a recent study across 48 species of the Jena Experiment, including most of our species but using plants without selection history in the Jena Experiment, plant–soil

feedbacks were found to be predominantly negative (Cortois et al. in revision), in line with our results for mixture types. We thus suggest that monoculture types have acquired their positive plant–soil feedbacks during the 8 years of selection in monoculture plots of the Jena Experiment and as a consequence of their good performance in monoculture have, in comparison to mixture types, less increased biodiversity and complementarity effects on soils with legacy.

Previous research has shown that individual plant performance can be affected by plant–soil feedbacks, which could promote coexistence in plant communities (Klironomos 2002). Such feedbacks can contribute to positive biodiversity–productivity relationships in grasslands (Kulmatiski et al. 2012). Here we have shown that diversity itself can act as a selection pressure to modify plant–soil feedbacks, with high-diversity environments selecting for plant genotypes/phenotypes susceptible to negative plant–soil feedbacks and low-diversity environments selecting for plants with the potential to benefit from positive plant–soil feedbacks. This adds to earlier findings that different levels of biodiversity can select for trait differences in plant species (Lipowsky et al. 2011) and that in particular high diversity environments can also select for increased niche complementarity via character displacement (Zuppinger-Dingley et al. 2014). It is conceivable that the increased performance of mixture-selected plants, mixture types, in mixtures on neutral soil is in part facilitated by an evolutionarily decreased pathogen defense, freeing up resources for enhanced growth (Bazzaz et al. 1987). Given the widely-acknowledged role of the growth–defense tradeoff in allocation of resources in plants (Coley et al. 1985, Herms and Mattson 1992, Fineblum and Rausher 1995) and of nutrients in leaves (Agrawal and Fishbein 2006), any process which reduces the allocation to defense should increase growth at the individual level and thus productivity at the community level.

344 The positive biodiversity effect on productivity that we observed in our plant
345 communities has been widely observed in grasslands (Balvanera et al. 2006, Isbell et al.
346 2009). However, here we could demonstrate for the first time that the biodiversity–
347 productivity relationship was influenced by plant-selection and soil-legacy effects within 8
348 years of selection, such that the selection within species in mixture when grown in
349 combination with the co-developed soil biota showed a particularly strong biodiversity
350 complementarity effect. The selection of the different phenotypes in mixtures vs.
351 monocultures may reflect different genotypes or epigenetic variation, or differential,
352 persistent maternal carry-over effects. Considering that the initial seed material was
353 genetically variable (Amuni Ghazzaoui, *personal communication July 2015*) and that all
354 plants were raised from seeds under common conditions, we suggest a sorting-out process of
355 different genotypes within sown communities in the Jena Experiment, aided perhaps by
356 recombination during pollination and seed production.

357 Thus, short-term plant evolutionary and soil community assembly processes may
358 interact in experimental communities and increase both their productivity and stability over
359 time due to enhanced complementarity effects (Isbell et al. 2009). Such processes may help
360 explain the increasing complementarity effects over time in many biodiversity experiments
361 (e.g. see Cardinale et al. 2007, Reich et al. 2012). In parallel with the increased
362 complementarity effect, the sampling effect decreased for plant communities composed of
363 mixture types and grown in soil with a mixture legacy. This is again consistent with previous
364 studies, which found that such sampling effects tend to decrease over time in mixture
365 communities (Cardinale et al. 2007, Isbell et al. 2009). Low and even negative sampling
366 effects for individuals selected in mixtures and planted in mixed test communities suggest
367 pathogen control of dominant species (Morris et al. 2007, Mordecai 2011) implying that
368 communities may be regulated in part by negative plant–soil feedbacks (Bever 1994, Mills

and Bever 1998). Negative sampling effects can also result when species with conservative growth strategies and low monoculture performance become competitively dominant in mixtures (Hooper and Dukes 2004). It is conceivable that such species (see above discussion of low-yielding species *Galium mollugo* and *Prunella vulgaris*) or such genotypes within species may be favored on soils with legacy.

The major new finding of the present study is that not only may selection in mixtures lead to increased complementarity effects, but in fact selection in monoculture may to a certain extent counteract the process of increasing biodiversity effects over time. This can occur if plant monoculture types develop an increased capability to benefit from mutualistic interactions with soil organisms accumulating in their rooting space over time. Co-adaptation between plants and soil organisms has been observed in other studies (Pregitzer et al. 2010). If plant-specific mutualists as well as antagonists become concentrated in monocultures, the low performance of monocultures may increase over time; indeed, in the Jena Experiment biodiversity effects started to decline again in recent years (E. De Luca, *personal communication March 2015*). Furthermore, experience from agriculture suggests that long-term cropping with the same plant species first leads to declining yields; however, in the long term, yield recovers, which has been explained by increased top-down control of plant pests and diseases (Weller et al. 2002).

Plant selection and soil legacy modify plant community resistance to invasion (hypothesis 2)

Increased stability of plant communities over time due to evolutionarily-increased complementarity effects may also be reflected in increased resistance to weed invasion. It is well known that high plant diversity can be associated with increased invasion resistance in plant communities (Naeem et al. 2000, Fargione et al. 2003, Jiang et al. 2007). In the present study we also found this effect and we were able relate the response to the higher plant biomass in pots planted with mixtures relative to pots planted with monocultures. Niche

complementarity in communities of high diversity can be a factor contributing to resistance to invasion (Shea and Chesson 2002, Theoharides and Dukes, 2007) by suppressing the growth of invasive species (Fargione et al. 2003, van Ruijven et al. 2003). As mixture types have been shown to be selected for increased complementarity via greater niche differentiation between species (Zuppinger-Dingley et al. 2014), the observed slight decrease in proportional weed biomass in pots of mixture types in mixtures (see Figure 4) may be indicative of reduced available "niche space".

However, contrary to the expectation that mixture types may have evolved narrower niches and thus should allow more weed invasion than monoculture types in monocultures, this was only the case on soil with legacy and not on neutral soil. Because the effect, that monocultures of mixture types growing on soil with legacy had particularly high weed invasion, could not be explained by the covariate community biomass of planted species, it might have directly been due to altered plant–soil feedbacks. Thus, we currently have no mechanistic explanations why mixture types, derived from plants that had been selected for 8 years in high-diversity treatments, had lower invasion resistance than monoculture types in monocultures on soils with legacy but not on soils without. To find such explanations, it would be necessary to assess the plant–soil interactions of mixture and monoculture types in more detail than was possible in the present study.

Conclusions

Our study suggests that in experimental plant communities of low vs. high diversity, differential selection of particular plant phenotypes and of particular communities of soil organisms leads to plant-selection and soil-legacy effects that may explain the increased biodiversity effects over time that are typically observed in biodiversity experiments. This was indicated by the increased complementarity and decreased sampling effects in communities of mixture types on soils with legacy, in comparison with neutral soils. Positive

complementarity and negative sampling effects also promote species coexistence and may thus enhance productivity as well as temporal stability of plant communities (Isbell et al. 2009). The results of this study emphasize the importance of time for community assembly and the development of biodiversity effects under experimental conditions (Reich et al. 2012, Kardol et al. 2013, Wolkovich et al. 2014). If similar processes occur in natural systems (Mittelbach and Schemske 2015), older communities may be more stable and have higher productivity than younger communities of similar species composition, with obvious consequences for ecosystem restoration. By understanding mechanisms whereby primary producers coexist, adapt to each other and their soil environment, and drive ecosystem productivity, we may be able to better predict the potential effects of species and genotype loss on the functioning of natural ecosystems.

Acknowledgements

We thank Daniel Trujillo Villegas, Leigh Oesch and student helpers for assistance with greenhouse and garden work. We also thank Theres Zwimpfer, Matthias Furler, René Husi and the gardeners of the Jena Experiment for technical assistance and Dave Hooper and an anonymous reviewer for very helpful comments on an earlier version of the manuscript. This study was supported by the Swiss National Science Foundation (grant number 130720 to B.S.) and the University Research Priority Program Global Change and Biodiversity of the University of Zurich. G.B. De Deyn acknowledges the NWO-ALW VIDI grant scheme for financial support.

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577 **Ecological archives:**

578 Appendices

579 **Appendix A**: Supplemental methods with experimental species combinations and the results
580 of elemental analysis of the soils used for the study.

581 **Appendix B**: Supplemental results with mixed-effects ANOVA for survival of all plant
582 individuals 15 weeks after planting.

583 **Table 1**: Results of mixed-effects ANOVA for the log-transformed community aboveground
584 biomass 15 weeks after transplanting plants into pots (numDF: degrees of freedom of term,
585 denDF: degrees of freedom of error term [which can be fractional in REML analysis], F:
586 variance ratio, *P*: error probability, VC: variance component, s.e.: standard error of variance
587 component).

Fixed terms	numDf	denDf	F	*P*
Planted diversity: planted monocultures vs. planted mixtures (PD)	1	22.1	6.38	**0.019**
Plant selection: monoculture vs. mixture (PS)	1	59.4	0.29	0.590
Soil legacy: live vs. sterilized inoculum (SL)	1	58.1	0.00	0.960
PD × PS	1	58.6	0.00	0.969
PD × SL	1	57.7	3.84	0.055
PS × SL	1	58.3	5.76	**0.020**
PD × PS × SL	1	57.8	1.49	0.227
Random terms	VC	s.e.		
Species combination (Sp.comb)	0.0056	0.0093		
Block (B)	0.5938	0.1921		
B × Sp.comb	0.0278	0.0196		
Sp.comb × PS × SL	0.0496	0.0250		
Residual	0.2200	0.0275		

588

589 **Table 2:** Results of mixed-effects ANOVA for the biodiversity net effect (untransformed), the
590 complementarity effect (square-root transformed), and the sampling effect (square-root
591 transformed; numDF: degrees of freedom of term, denDF: degrees of freedom of error term
592 [which can be fractional in REML analysis], F: variance ratio, *P*: error probability, VC:
593 variance component, s.e.: standard error of variance component).

Net Effect				
Fixed terms	numDf	denDf	F	*P*
Plant selection: monoculture vs. mixture (PS)	1	160.3	0.89	0.346
Soil legacy: live vs. sterilized inoculum (SL)	1	157.1	3.19	0.076
PS × SL	1	157.1	0.14	0.710
Random terms	VC	s.e.		
Species combination	159.1	64.9		
Residual	202.1	22.8		
Complementarity Effect				
Fixed terms	numDf	denDf	F	*P*
Plant selection: monoculture vs. mixture (PS)	1	161.2	1.69	0.196
Soil legacy: live vs. sterilized inoculum (SL)	1	157.2	8.09	**0.005**
PS × SL	1	157.2	3.98	**0.048**
Random terms	VC	s.e.		
Species combination	4.161	1.781		
Residual	7.771	0.877		
Sampling Effect				
Fixed terms	numDf	denDf	F	*P*
Plant selection: monoculture vs. mixture (PS)	1	14.1	0.13	0.726
Soil legacy: live vs. sterilized inoculum (SL)	1	14.7	6.74	**0.021**

PS × SL	1	137.4	7.85	**0.006**
Random terms	VC	s.e.		
Species combination (Sp.comb)	1.314	0.974		
[i]Sp.comb × PS	0.096	0.520		
[i]Sp.comb × SL	0.722	0.746		
Residual	7.014	0.872		

i) these random effects were bound in the final models for net and complementarity effect and therefore excluded.

595 **Table 3**: Results of mixed-effects ANOVA for soil-feedbacks calculated using the log-ratio of
596 total plant aboveground biomass of pots inoculated with live vs. sterilized soil from the Jena
597 biodiversity experiment (numDF: degrees of freedom of term, denDF: degrees of freedom of
598 error term [which can be fractional in REML analysis], F: variance ratio, *P*: error probability,
599 VC: variance component, s.e.: standard error of variance component).

Fixed terms	numDf	denDf	F	600 *P*
Planted diversity: planted monocultures vs. planted mixtures (PD)	1	21.2	1.79	601 0.195
Plant selection: monoculture vs. mixture (PS)	1	113.9	10.27	**0.002**
PD × PS	1	112.0	4.06	**0.046**
Random terms	VC	s.e.		
Species combination	0.070	0.049		
Residual	0.494	0.067		

602 **Table 4**: Results of mixed-effects ANOVA for the proportion of weeds in the total
603 aboveground biomass (planted species + weeds) in a pot (numDF: degrees of freedom of
604 term, denDF: degrees of freedom of error term [which can be fractional in REML analysis], F:
605 variance ratio, *P*: error probability, VC: variance component, s.e.: standard error of variance
606 component).

Fixed terms	numDf	denDf	F	*P*
Planted diversity: planted monocultures vs. planted mixtures (PD)	1	22.1	7.59	**0.012**
Plant selection: monoculture vs. mixture (PS)	1	59.0	0.36	0.550
Soil legacy: live vs. sterilized inoculum (SL)	1	22.1	0.88	0.357
PD × PS	1	58.6	0.12	0.728
PD × SL	1	22.0	3.15	0.090
PS × SL	1	33.2	2.45	0.127
PD × PS × SL	1	32.9	6.22	**0.018**
Random terms	VC	s.e.		
Species combination (Sp.comb)	0.0127	0.0044		
Sp.comb × SL	0.0013	0.0009		
Sp.comb × SL × PS	0.0017	0.0097		
Sp.comb × PS × Block	0.0024	0.0006		
Residual	0.0034	0.0005		

607

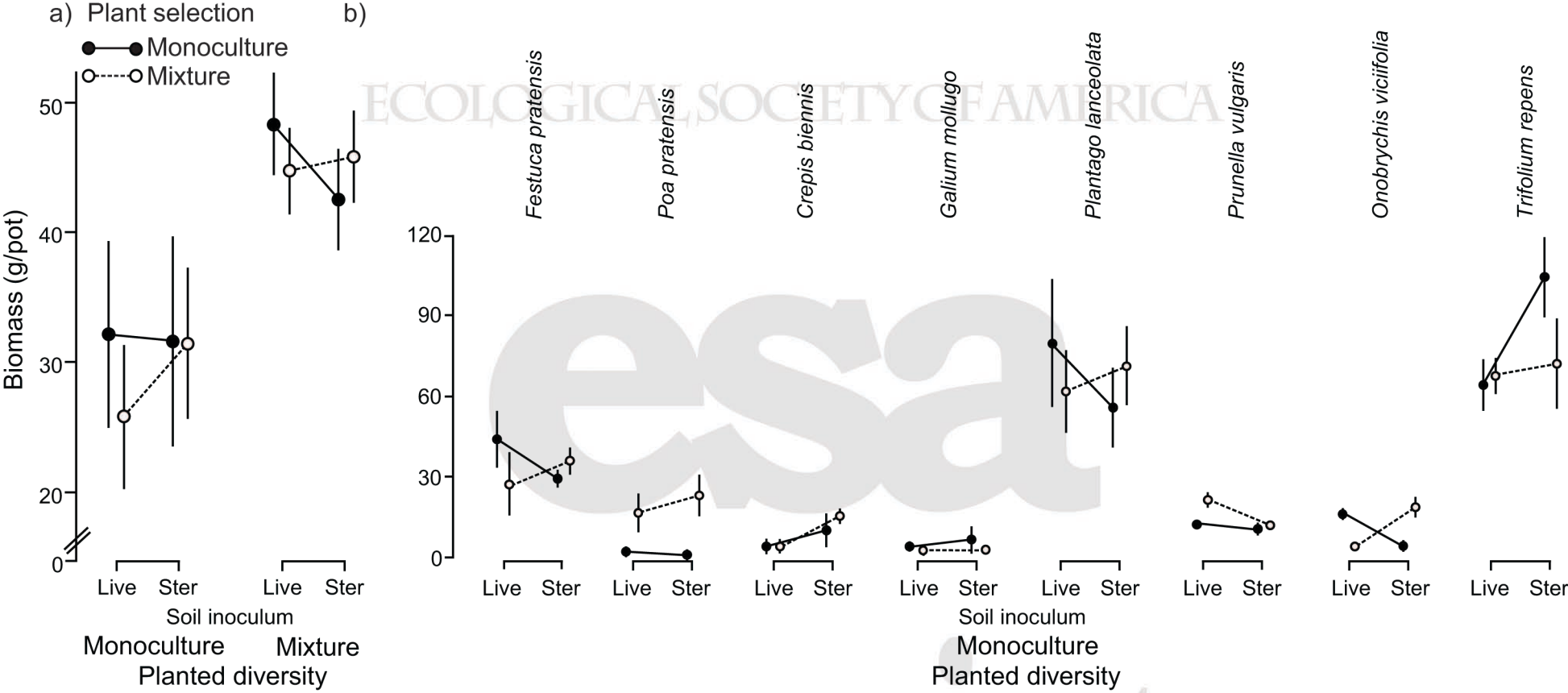
608 **Fig. 1:** a) Mean aboveground community biomass of progeny of plants selected in
609 monocultures vs. mixtures in a biodiversity experiment in Jena, Germany (plant-selection
610 treatment) and grown in monoculture vs. mixture (planted diversity treatment) in pots
611 inoculated with live vs. sterilized soil from the same biodiversity experiment (soil-legacy
612 treatment; bars are means ± 1 SEM). b) As in left side panel a) separated for the 8 species
613 grown in monoculture; the species are arranged according to functional groups from left to
614 right: grasses, tall herbs, small herbs, legumes.

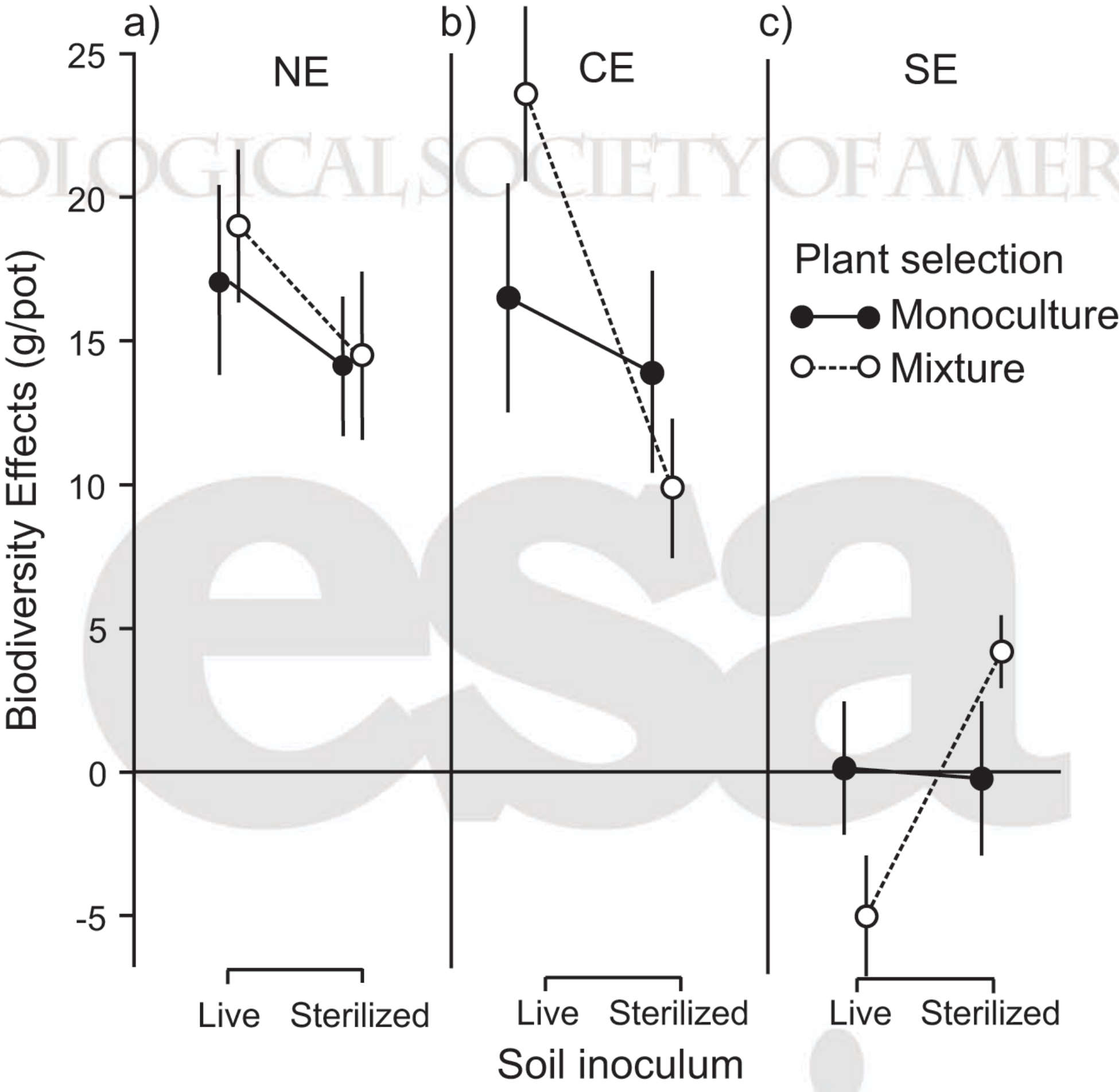
615 **Fig. 2:** Biodiversity effects were assessed by additive partitioning of a) the net effect (NE)
616 into b) complementarity effect (CE) and c) sampling effect (SE) for progeny of plants selected
617 in monocultures vs. mixtures in a biodiversity experiment in Jena, Germany (plant-selection
618 treatment) and grown in pots inoculated with live vs. sterilized soil from the same biodiversity
619 experiment (soil-legacy treatment; bars are means ± 1 SEM).

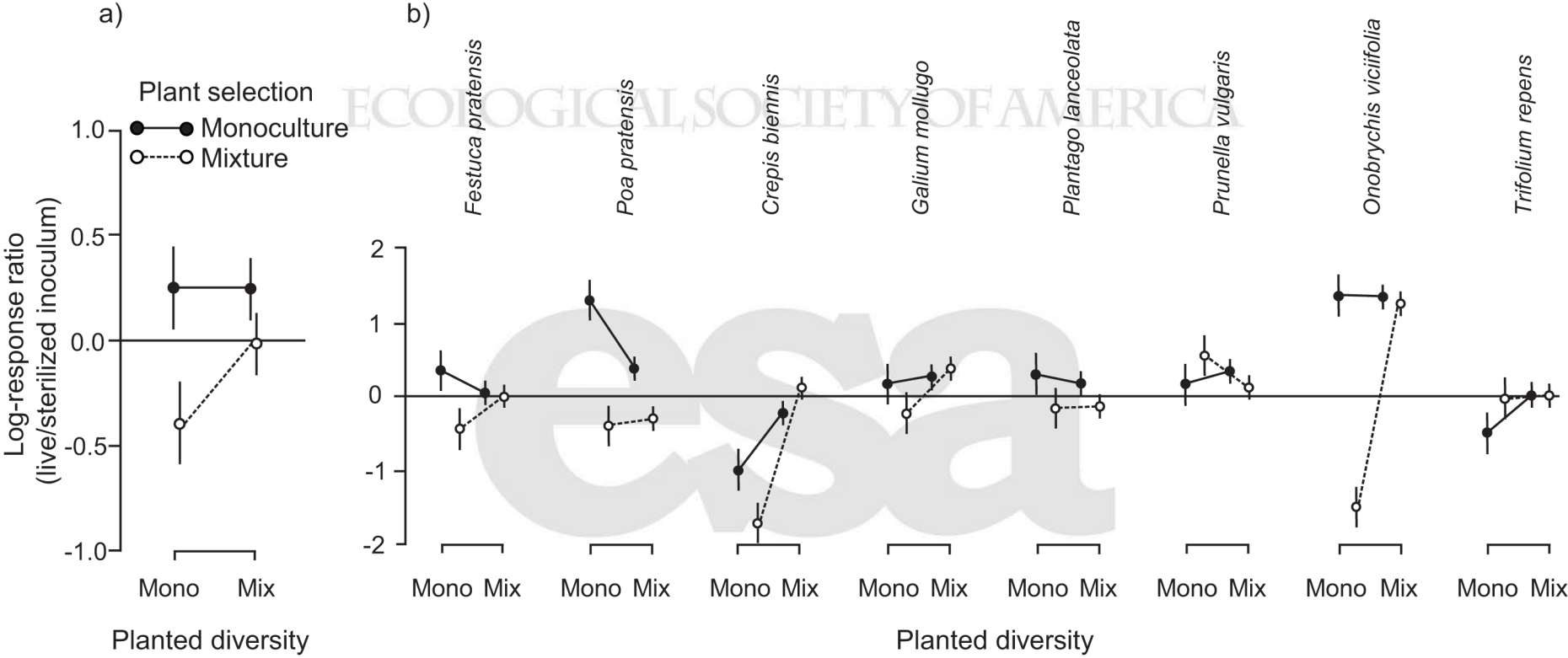
620 **Fig. 3**: a) Values for plant–soil feedbacks calculated as the log-ratio of aboveground
621 community biomass of plants in pots inoculated with live vs. sterilized soil (soil-legacy
622 treatment) for progeny of plants selected in monocultures vs. mixtures in a biodiversity
623 experiment in Jena, Germany (plant-selection treatment) and grown in monoculture vs.
624 mixture (planted diversity treatment; bars are means ± 1 SEM). Negative values indicate
625 negative soil feedbacks and positive values indicate positive soil feedbacks. b) The increased
626 negative plant–soil feedback for progeny of plants selected in mixtures and then grown in
627 monoculture as compared with progeny of plants selected in monoculture and then grown in
628 monoculture was observed in most of the plant species (values for soil feedbacks calculated as
629 in Fig. 3a but with community biomass separated into species). In b) the species are arranged
630 according to functional groups from left to right: grasses, tall herbs, small herbs, legumes.

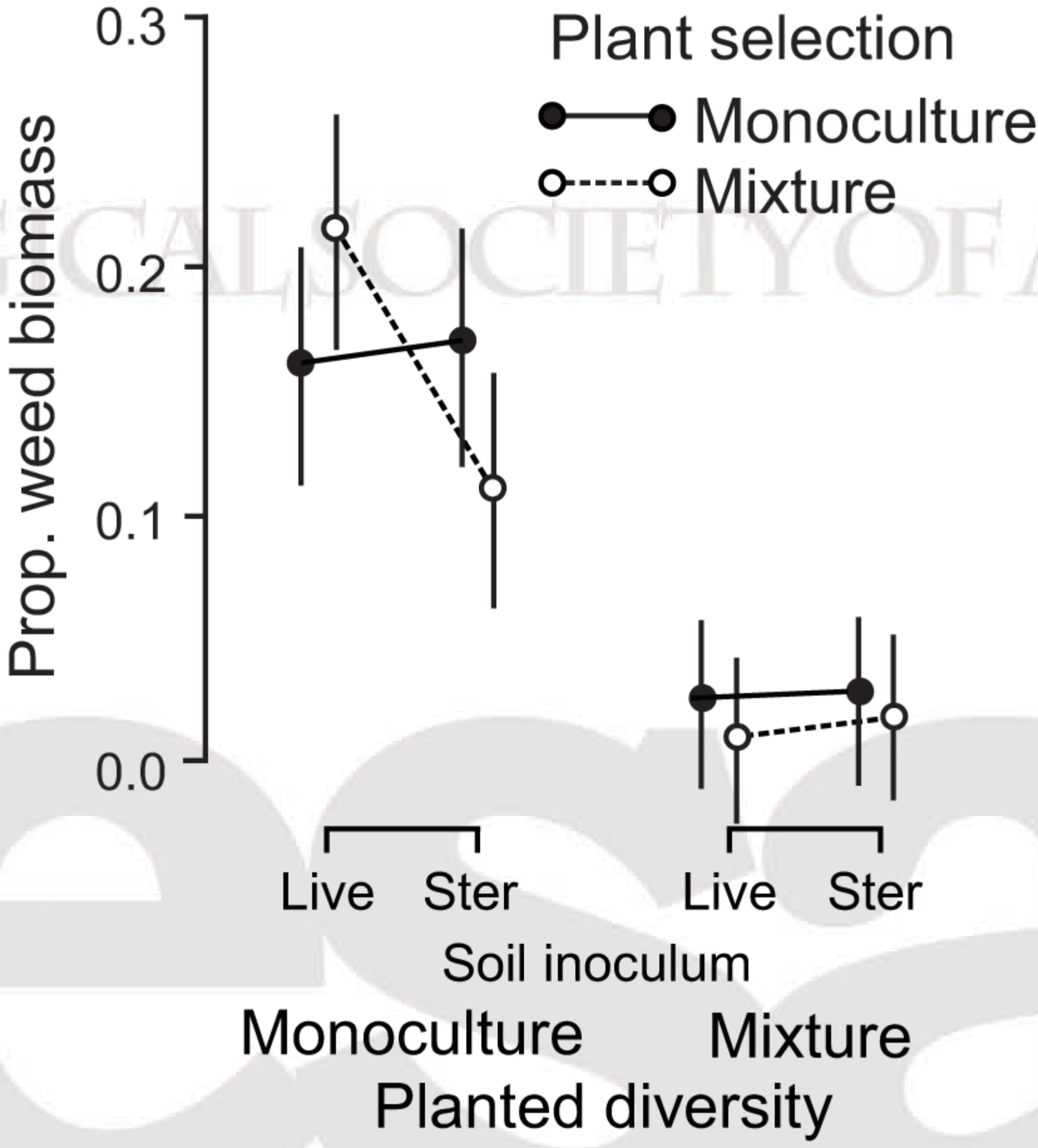
631 **Figure 4**: Proportion of weeds in the total aboveground biomass (planted species + weeds) in
632 pots planted with progeny of plants selected in monocultures vs. mixtures in a biodiversity

633 experiment in Jena, Germany (plant-selection treatment) and grown in monoculture vs.
634 mixture (planted diversity treatment) inoculated with live vs. sterilized soil from the same
635 biodiversity experiment (soil-legacy treatment; bars are means ± 1 SEM).


a) Plant selection
b)
Monoculture
Mixture
Biomass (g/pot)
50
40
30
20
0
120
90
60
30
0
Live
Ster
Soil inoculum
Monoculture
Mixture
Planted diversity
Festuca pratensis
Poa pratensis
Crepis biennis
Galium mollugo
Plantago lanceolata
Prunella vulgaris
Onobrychis viciifolia
Trifolium repens


a)
NE
b)
CE
c)
SE
25
20
15
10
5
0
-5
Biodiversity Effects (g/pot)
Plant selection
Monoculture
Mixture
Live
Sterilized
Live
Sterilized
Live
Sterilized
Soil inoculum


a)
b)
Plant selection
Monoculture
Mixture
Log-response ratio
(live/sterilized inoculum)
1.0
0.5
0.0
-0.5
-1.0
Mono
Mix
Planted diversity
Festuca pratensis
Poa pratensis
Crepis biennis
Galium mollugo
Plantago lanceolata
Prunella vulgaris
Onobrychis viciifolia
Trifolium repens
2
1
0
-1
-2
Mono Mix
Planted diversity


Plant selection
Monoculture
Mixture
Prop. weed biomass
0.3
0.2
0.1
0.0
Live
Ster
Live
Ster
Soil inoculum
Monoculture
Mixture
Planted diversity